

CHEMOTAXONOMIC ASPECTS OF THE BUCHU SPECIES *AGATHOSMA BETULINA* (PILLANS) AND *AGATHOSMA CRENULATA* (PILLANS) FROM LOCAL PLANTINGS

K.L.J. BLOMMAERT AND E. BARTEL¹

(Fruit and Fruit Technology Research Institute, Stellenbosch)

ABSTRACT

Measurements of leaf form from buchu plants collected in local plantings indicated that this criterion, the only taxonomic basis for distinguishing the two species, is a reliable method of identification but this does not hold true for hybrid buchu. G.L.C. and U.V. analysis of buchu oils showed that the presence of a relatively large percentage of ψ -diosphenol and diosphenol in *A. betulina* leaf oil only, is a valid criterion for botanically separating the two species. The analyses also showed that there is a wide variation in the percentage composition of the major components and in the percentage of oil obtained from different buchu plants.

UITTREKSEL

CHEMOTAKSONOMIESE IDENTIFIKASIE VAN DIE BOEGOE SPESIES *AGATHOSMA BETULINA* (PILLANS) EN *AGATHOSMA CRENULATA* (PILLANS) UIT PLAASLIKE AANPLANTINGS

Boegoe-monsters is in drie plaaslike aanplantings versamel en visueel op grond van blaarvorm as rondebelaar, ovaalblaar en basters geklassifiseer. Die blaarmonsters is in die laboratorium gedroog en die oliedistillaat van elk, gaschromatografies en massaspektrometries analiseer; die U.V. spektra is ook bepaal. Die lengte/breedte verhouding van afsonderlike blare per monster is ook bereken.

Die lengte/breedte verhouding van die blare is 'n betroubare maatstaf om die twee boegoe spesies te onderskei maar dit geld nie vir baster boegoe nie. Slegs rondebelaar boegoe (*A. betulina*) bevat die komponente ψ -diosfenol en diosfenol in redelike hoeveelheid en toon 'n U.V. piek by 272 nm. Ovaalblaar-boegoe (*A. crenulata*) toon 'n kenmerkende piek by 252 nm, te wyte aan pulegon. Die teenwoordigheid van die twee diosfenole in rondebelaar boegoe is blykbaar 'n betroubare maatstaf om die twee spesies botanies te onderskei, maar dit geld nie in die geval van basterboegoe nie, wat ook die twee diosfenole, maar in kleiner hoeveelhede, mag bevat. Die hoof oliebestanddele van albei spesies is kwalitatief dieselfde maar toon groot kwantitatiewe verskille. Daar is ook relatief groot verskille in die persentasie olie wat van individuele plante verkry is.

INTRODUCTION

The natural habitat of the two commercially important buchu species *Agathosma betulina* and *A. crenulata* roughly extends from the Clanwilliam to Tulbagh, Paarl and Riversdale areas respectively. Taxonomically they are distinguished on the basis of leaf form only (Pillans, 1950). *A. betulina* is characterised by a smaller and rounded leaf shape as opposed to *A. crenulata* which has a larger oval-shaped leaf. This has been the basis of visual identification

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¹University of Stellenbosch.

used by growers and exporters and the two species are commonly known as round-leaf and oval-leaf buchu. However, since the establishment of buchu plantings in various parts of the Western Cape, a great deal of confusion as to the identity of these plants has arisen. Apparently a considerable amount of hybridisation has taken place over the years whenever the two species were interplanted and new plantings were established from seed collected in such plantings. Intermediate leaf forms are very common and the grower is faced with the problem of distinguishing between the two species. This is particularly important because overseas buyers prefer the round-leaf species which also fetches higher prices.

From a chemical point of view it has been known for some time that the essential oil produced from the dried leaves of *A. betulina* contains a considerable amount of diosphenol, the so-called buchu camphor. It has been shown by Fluck, Mitchell and Perry (1961) that an isomer of diosphenol, ψ -diosphenol, also occurs in *A. betulina* oil while *A. crenulata* oil contains only trace amounts of both components. The ultra-violet absorption spectrum of diosphenol and ψ -diosphenol are identical showing an absorbance peak at 272 nm (Fluck *et al.*, 1961). They found that the oil of *A. betulina* also shows this characteristic peak thus providing a method of identifying this buchu species.

In an effort to identify the two buchu species, leaf samples were collected from established plantings and analyses of the oil distillates carried out by U.V. spectrophotometry and gas-liquid chromatography. Length/width ratios of leaf samples were also determined as a possible criterion of identity.

MATERIAL AND METHODS

Buchu leaf samples were collected during the summer from three plantings of growers in the Stellenbosch and Paarl area. The individual plants were visually classified as round, oval or intermediate (hybrid) according to leaf shape. The leaves were dried in an oven at 25°C and stored in air-tight glass containers at room temperature until analysed. The length and width of 20 leaves per plant were measured.

A ten gram leaf sample per plant to which 50 ml distilled water had been added, was water distilled for three hours in an all glass Karlsruhe* apparatus and the volume of oil obtained per sample was recorded. The U.V. absorption spectra of 2 mg of oil, taken up in 50 ml ethanol, were recorded on a Pye-Unicam spectrophotometer. The G.L.C. analyses were carried out with a Perkin-Elmer, Model-990 gaschromatograph. The recording conditions for the latter were as follows: Sample volume 1 μ l; injector temperature, 200°C,

*Bender and Hobein, Karlsruhe, West Germany.

detector temperature 210°C; 38 cm × 3,1 mm o.d. stainless steel column packed with 10% Carbowax 20 M on Chromosorb W/AW DMCS, 80–100 mesh; carrier gas-N₂; programme, 50°C to 150°C at 1°C per minute. The analyses were programmed to separate the main buchu oil components only. The relative percentage of each component per sample was estimated from the peak heights measured from a common base line. Peak height versus peak area *i.e.* peak height times width at half height showed such good correlation that it was possible to use peak height only. Identification of the components were made by direct coupling of the gas chromatograph to an AEI MS 902 mass spectrometer through a silicone membrane separator.

RESULTS

The results of analyses of buchu oil samples distilled from 24 individual plants are given in Table 1. It appears that pulegone is probably the most abundant constituent of the oil of both species followed by menthone and limonene which occur in approximately equal amounts. There is a striking variation between plants with regard to the percentage composition of the major components as shown by the C.V. values in Table 1. This has not been reported before as earlier analyses were apparently based on oil samples of commercial origin which are normally distilled from composite leaf batches exported from this country. The results confirm that ψ -diosphenol and diosphenol occur in round-leaf buchu only, whereas the other major components are qualitatively similar (See Fig. 1 and 2). The percentage ψ -diosphenol plus diosphenol (buchu camphor) in round-leaf buchu oil varies considerably and

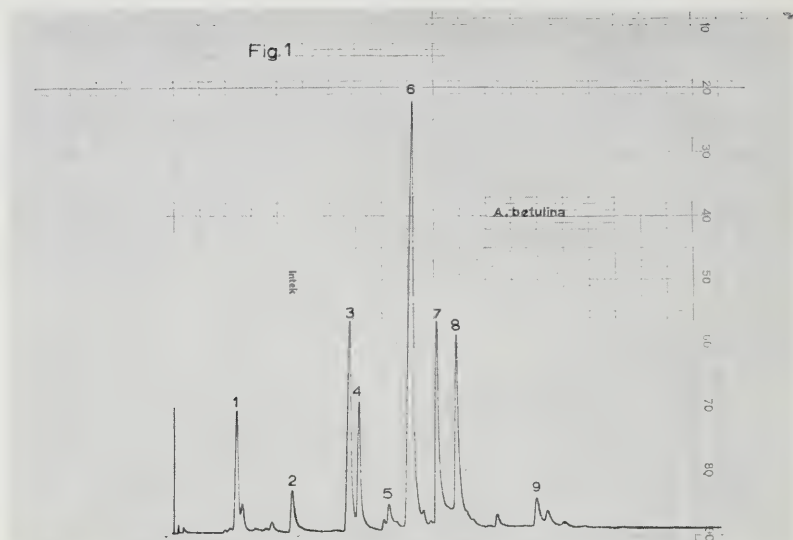
TABLE 1

Average values and coefficient of variation (CV) for the major oil components, oil content and length/width ratios of dried leaves from individual *A. betulina* (round leaf), *A. crenulata* (oval leaf) and hybrid buchu plants. (Transformed data (Arcsine) are given in brackets.)

Component	Round leaf*		Oval leaf*		Hybrid**		LSD
	%	CV	%	CV	%	CV	(p=0,05)
Limonene	15,75	50,03	11,80	36,69	12,38	21,26	ns
Unidentified	1,03	69,90	11,72	52,99	7,58	46,31	4,99
Menthone	21,32	27,30	12,65	24,90	14,48	29,70	5,39
Iso-menthone	15,12	39,68	7,72	27,59	8,42	34,44	4,50
Iso-pulegone	2,45	65,31	4,55	6,37	4,42	21,04	1,25
Pulegone	31,65	42,34	46,95	7,03	45,56	15,58	10,17
ψ -Diosphenol	5,08	95,08	0,00	0,00	1,93	227,98	7,27
	(11,96)		(0,00)		(3,70)		
Diosphenol	4,38	100,00	0,00	0,00	1,79	243,08	7,09
	(11,02)		(0,00)		(2,10)		
8-Acetylthiomenthanone-3	3,15	77,78	4,65	21,19	3,74	27,54	ns
% oil	2,53	34,78	2,38	22,27	2,43	25,10	ns
l/w Ratio	1,98	6,06	2,37	4,22	2,22	9,91	0,20

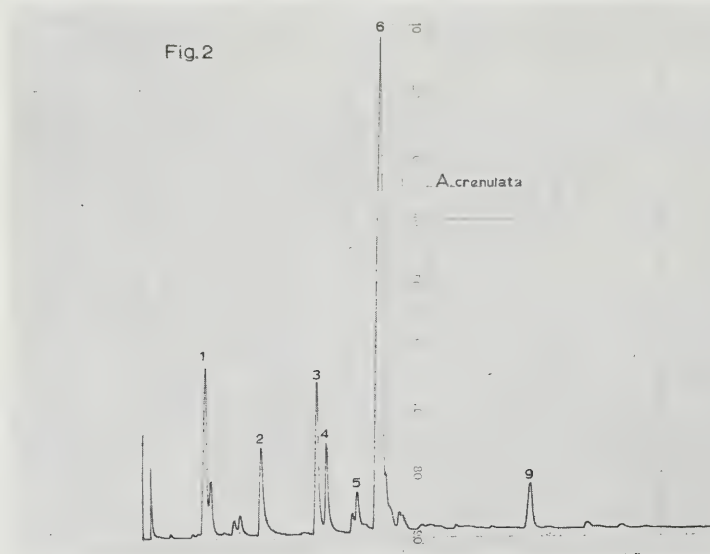
*Mean of six plants

**Mean of twelve plants



Representative gaschromatographs of *A. betulina* (Fig. 1) and *A. crenulata* (Fig. 2) leaf oils. Components:

1. Limonene, 2. Unidentified, 3. Menthone, 4. Iso-menthone, 5. Iso-pulegone, 6. Pulegone,
7. ψ -Diosphenol, 8. Diosphenol, 9. 8-Acetylthiomenthانون-3.



ranges from 28 % to 2,7 %. The two buchu species were also compared in terms of the new sulphur-containing compound, 8-acetyl-thiomenthanone-3, which is present in comparable concentration to the other major components. It appeared on the chromatogram at a retention time much higher than any other component hitherto identified. Identification was done by both mass spectrometer and NMR spectrometer. However, before this investigation had been completed a copy of a report by Kaiser, Lamparsky and Schudel (1973), was received in which the identification of this particular component had already been reported.

The U.V. absorption peaks also appear to be reliable criteria for identifying the two buchu species. All oil samples containing both diosphenols show a characteristic peak at 272 μm while the remainder show a peak at 252 μm . The latter is identical with that of pulegone which is the major oil constituent of both buchu species. The large variation in oil content between plants ranging from 4,0 % to 1,4 % is also of particular significance. The ratios of leaf length/width of round-leaf buchu significantly differed from those of both oval-leaf and hybrid buchu. For the latter two groups however, these ratios were not significantly different.

CONCLUSIONS

The taxonomic classification of the two buchu species *A. betulina* and *A. crenulata* is almost solely based on leaf form. In their natural habitat this criterion is probably valid. However, in present day plantings in which many progeny probably originated from hybrid seed, distinction of the two species on the basis of leaf form is often difficult. The results indicate that the ratios of leaf length/width is a most useful criterion for identifying the two buchu species. However such distinction is not valid between oval leaf and hybrid buchu and this is supported by chemical analyses of the oil indicating that hybrid buchu contains all the major components including the two diosphenols although the latter occur in considerably lower concentration. It is generally accepted that the qualitative composition of essential oils such as buchu leaf oil, is a specific property of the plant species in question although considerable quantitative differences may occur between particular individuals (Swain, 1966). In the case of the two buchu species therefore, it would appear that the most reliable criterion to identify round-leaf and oval-leaf buchu is the presence of ψ -diosphenol and diosphenol in the former. On the other hand it is also well-known that alterations in chemical components may often fall under specific genetic control and consequently hybridisation between the two buchu species could have led to variation in oil composition.

Commercially, buchu leaf oil is mainly used in the flavour and pharmaceutical industries and overseas buyers have always preferred the oil from round-leaf buchu probably because of its so-called buchu camphor content. It is not clear

whether the presence of the latter is important *per se* or is merely used to distinguish oil derived from the two species. The present analyses clearly show that in future it will be important to identify and propagate only those plants with a high oil content and in the case of round-leaf buchu, high diosphenol content as well. In this way, a more standardised product of high quality could eventually be made available to buyers and consumers. Several trials at this Institute have shown that buchu plants cannot be propagated vegetatively by cuttings. It will therefore be necessary to collect seed from chemically selected plants and propagate the progeny in isolated localities as a source of seed which can be made available to growers.

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